Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers) II.

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ABSTRACT

Populations of *Helianthus annuus* L., ranging from eastern Oklahoma to North Dakota, to coastal southern California were sampled and the yields of total hydrocarbons (HC) from leaves determined. The highest yielding populations were in the Texas Panhandle (6.0 - 7.99%) and the lowest yields were in Camp Verde, AZ, NM mountains, Bozeman, MT, and ND - MN. Medium-high yields were found in northern UT and southern ID. Three populations near Waco, TX had large yield differences ranging from 4.9 to 6.2%, but a fourth population had a low 3.6%. Some native populations were contaminated by germplasm from cultivated sunflowers and these populations had very low yields (2.6 - 3.6%). Population variability in HC yields varied geographically and also between nearby populations, suggesting the micro-habitat environments are important as well as limited genetic population size. Published on-line www.phytologia.org *Phytologia 100(2): 153-160 (Jun 22, 2018).* ISSN 030319430.

KEY WORDS: *Helianthus annuus,* Sunflower, geographic variation in leaf hydrocarbon yields.

Adams and Seiler (1984) surveyed 39 taxa of sunflowers for their cyclohexane (hydrocarbon) and methanol (resins) concentrations. The highest cyclohexane (bio-crude) yielding taxa were *H. agrestis*, an annual, Bradenton, FL (7.38%) and *H. annuus*, Winton, OK (7.09%). Adams et al. (1986) screened 614 taxa from the western US for their hydrocarbon (hexane soluble) and resin (methanol soluble) yields. They reported 2 plants of *H. annuus* from Idaho with 8.71% and 9.39% hydrocarbon yields.

Seiler, Carr and Bagby (1991) reported on 28 *Helianthus* taxa for their yields of oil, polyphenols, protein and rubber. The rubber was found to be of lower molecular weight than *Hevea* rubber, but still appeared to be useful as a plasticizing additive and for coatings the inside of pipes and containers.

Yields of natural rubber has recently been reported for *H. annuus* (Pearson et al. (2010a) that ranged from 0.9% to 1.7% rubber in cultivated sunflower cultivars (Pearson et al. 2010b, Fig. 4,).

Most recently, Adams et al. (2018) reported on geographical variation in natural rubber yields in *H. annuus*. They found considerable variation in yields of natural rubber with the highest yielding populations at Mill Creek, UT and in the Waco, TX area. These high yielding populations are in very different eco-systems with different climates and soils. The rubber concentrations in adjacent plants sometimes varied from none to 16 mg/g.

Recently, Adams et al. (2017a) reported on hydrocarbon yields (HC) from a large area from Oklahoma to southern California. This report is a continuation of that survey of HC in sunflowers as we focus on basic research on *H. annuus* (Adams and TeBeest, 2016; Adams, et al. 2016).

MATERIALS AND METHODS

Population locations - see Appendix I.

The lowest growing, non-yellowed, 8 mature leaves were collected at stage R 5.1-5.3 when the first flower head opened with mature rays. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

Leaves were ground in a coffee mill (1mm). 3 g of air dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

RESULTS

The yields of hydrocarbons (HC) by population are given in Table 1. The highest yield (8.60%) was from Gruver, TX followed by Lake Tanglewood, TX (8.47%) in the Texas Panhandle. The lowest yield was from Woodward, OK (2.62%), Eagle Nest, NM (2.62%) and Clay county, MN (2.61%) followed by cultivated sunflowers (Oslo, TX)(3.20%). The Woodward population had smooth leaves as found in cultivated sunflowers. The plants and leaves were very large, although the heads were small. It appears that the Woodward population was a product of crosses between native and cultivated sunflowers and this resulted in the very low oil yield.

To visualize the variation in HC yields, the means were contour mapped (Fig. 1). Notice that the highest yields are in the Texas Panhandle. The west (EN, AZ) is region of low yields (Fig.1). A mosiac pattern of HC yields was found in sw Utah with low yield at KU (Kolob Canyon, 3.77%), but a high yield about 20 mi away at Leeds, UT (LU, 7.01%, Fig. 1). Another area of low yields is in North Dakota - Minnesota (GN, 3.23%, and CM, 2.1.6%, Fig. 1). These and the WO (Woodward, OK) are likely of hybrid origin between native and cultivated sunflowers. The southern Idaho - northern Utah area had medium - high yields. Of interest are the four populations near Waco, TX (MC, FC, LC, HC) that have 6.2, 5.3, 3.6, and 4.9% yields in a very small area. Adams et al. (2017b) examined the HC yields from naturally grown parents vs. green house grown progeny and reported much lower HC yields in greenhouse grown progeny compared to the parents growing under harsh environmental conditions. They concluded that up to half the HC production in nature is induced by insect damage and harsh environmental conditions. It may be that HC can be induced by chemicals sprayed on crops. It is very clear, from their greenhouse grown plants that high yields are not constitutive in *H. annuus*, but, rather, are induced by some factor not present in the greenhouse.

Adams et al. (2017a) found that the correlation between % yield and leaf weight as only r = 0.18 (highly significantly different from zero, df = 327). But the correlation accounted for only 3.24% (r^2) of the variance. They concluded that breeding for both increased % yields of HC and biomass seems feasible.

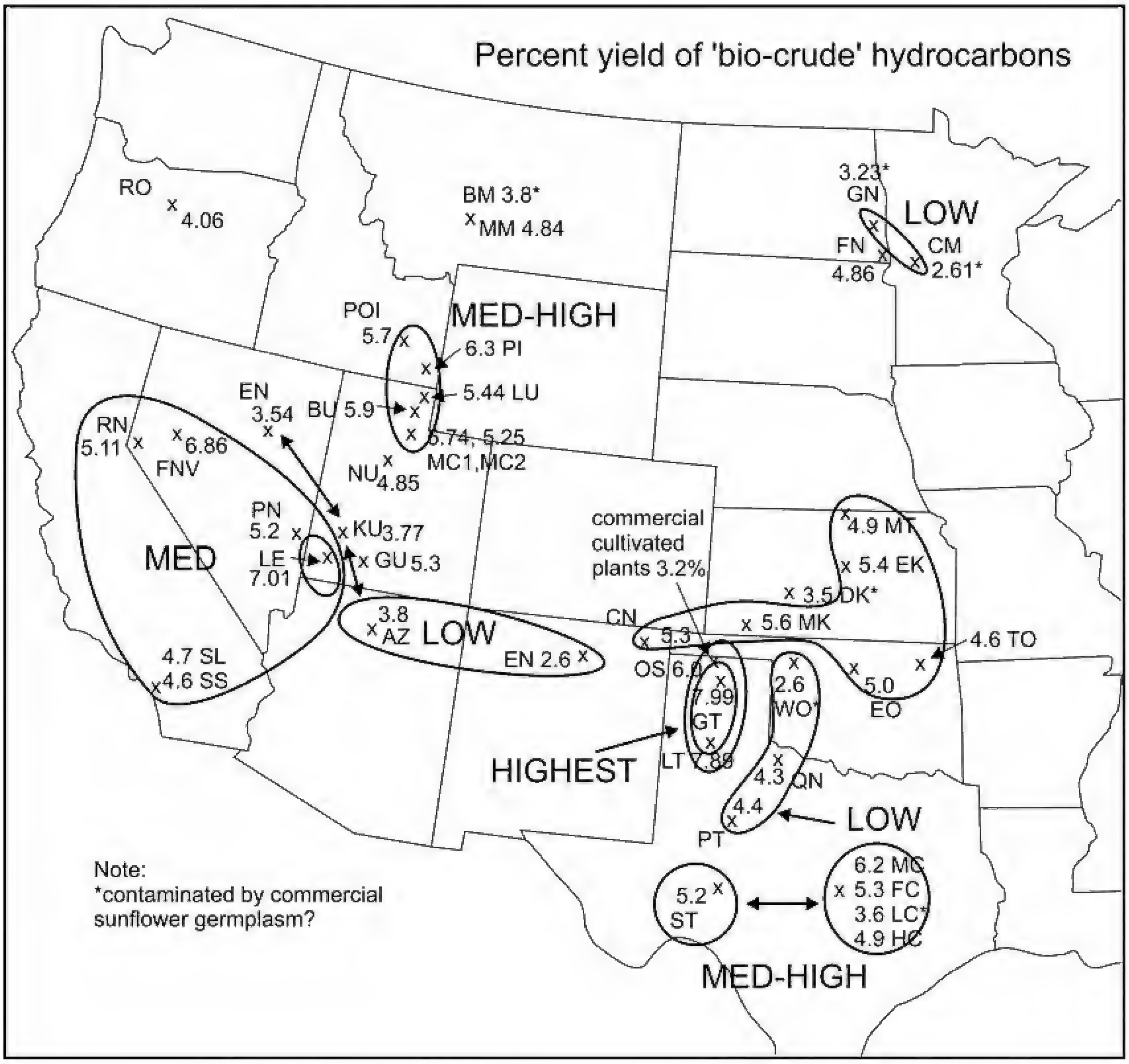

Figure 1. Geographic variation in % yields of HC by population. The asterisk (*) at the WO, LC, CM, GN, BM populations indicates that these population may be of hybrid origin between native and cultivated sunflowers.

The variability of yields by population is mapped in Figure 2. Population variability in HC yields varied geographically and also between adjacent populations, suggesting the micro-habitat environments are important as well as limited genetic population size. Some of the least variable populations were in North Dakota (FN, GN) and Minnesota (CM, Fig. 2). These populations appear to be introgressed from cultivated sunflowers, so that may explain the low variation.

Clearly the most unusual situation was the Waco, TX area where 4 nearby populations (MC, FC, LC, HC, Fig. 2) showed very small to large amounts of variation in their HC yields. MT (Montrose, KS) was from only 3 cultivated plants raised from seed, so its small variability may be just by chance.

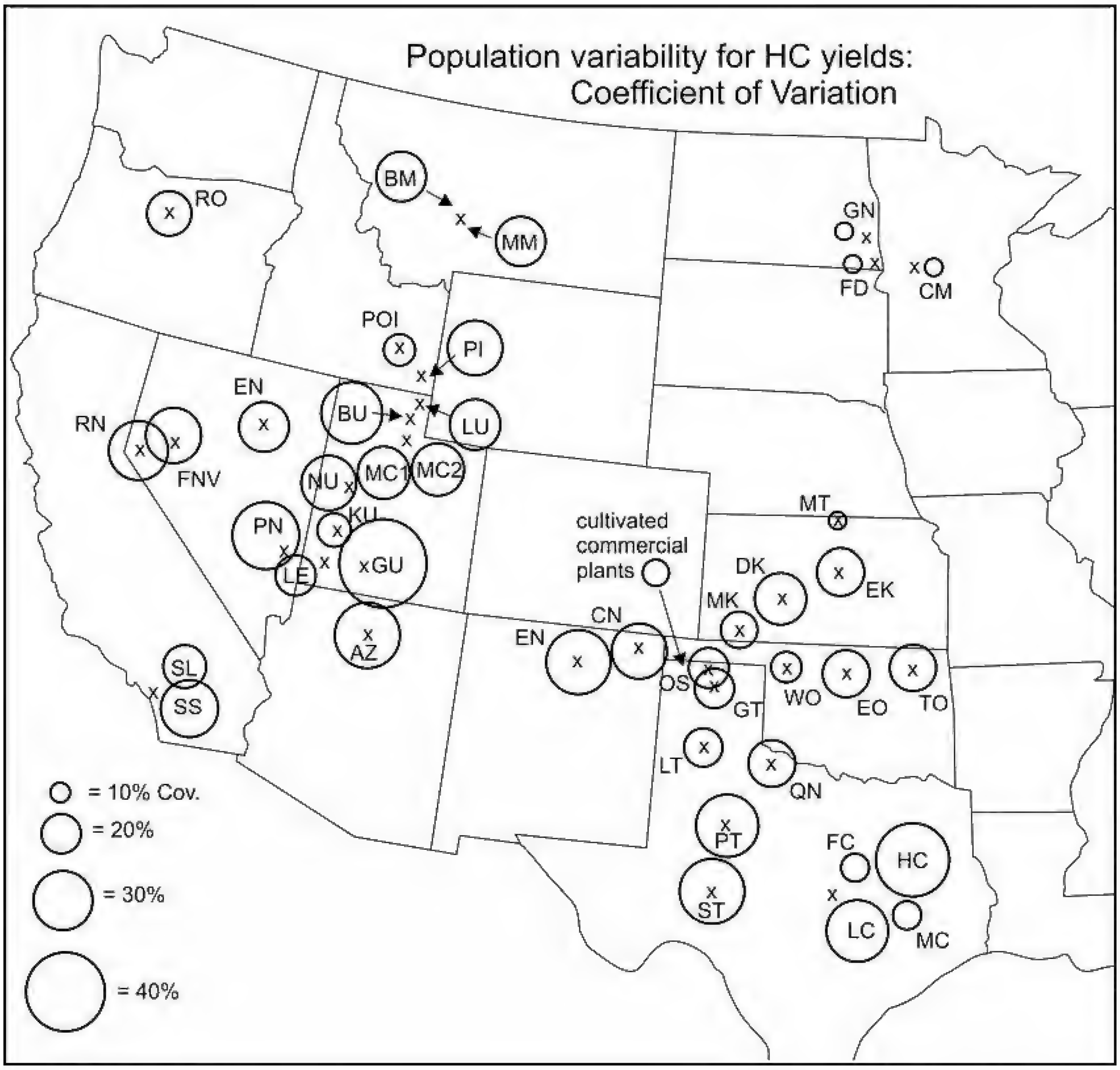

Figure 2. Population variability (coefficient of variation in HC yields) for the populations sampled. The size (diameter) of the circles is proportional to their coefficient of variation.

The total yields of HC per the weight of 8 mature leaves is a measure of the grams of HC per plant (likely larger than if the entire plant were extracted). In Table 1, the yields range from 0.03 g/ 8 mature leaves (Bozeman, MT) to 1.428 g (Gruver, TX). The highest yields were in the Texas Panhandle (1.20 g - 1.43 g) and Ellsworth, KS (1.0 g) and Enid OK (0.88 g) (Fig. 3). The lowest yields were in the southwestern United States. Note the difference between the San Diego, large leaves (SL, .43 g) and small leaves (SS, .27 g). These are plants collected from the same population. Recall that the % yields were quite similar (Table 1, SS, 4.59%; SL, 4.68%).

The northern-most populations in Montana, North Dakota and Minnesota were generally low in HC yields and that may reflect introgression from cultivated sunflowers (Fig. 3).

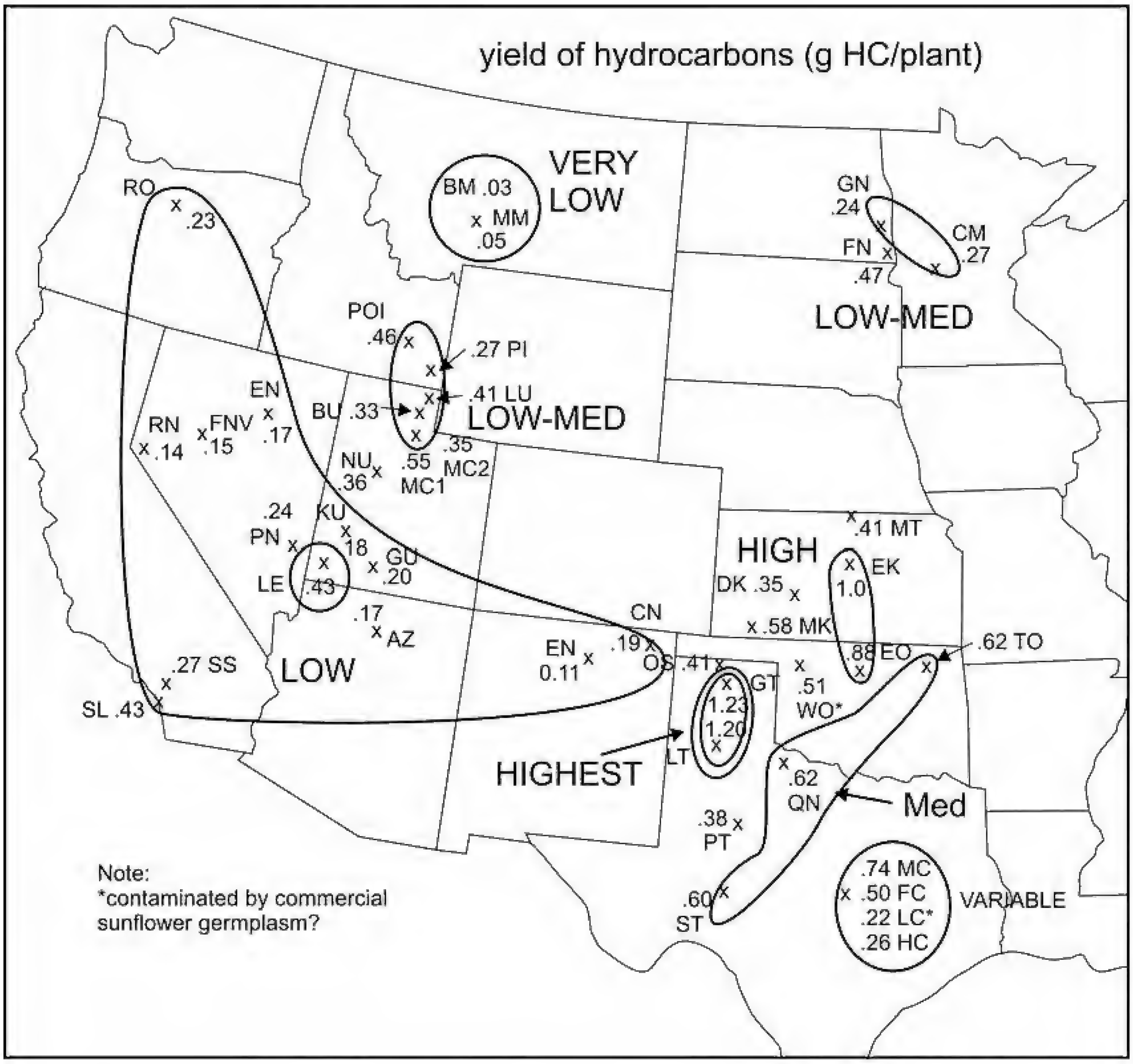

Figure 3. Geographic variation in the HC yields (g/ weight of 8 mature, dried leaves, basis).

This study revealed that the range of variation in HC yields of native sunflowers is quite large, from 1.0 to 12.63%. It is remarkable to find such a wide range, but indicates the potential of *H. annuus* to produce copious amounts of hydrocarbons for use as fuel and in the petro-chemical industry. Many of the highest yielding plants were severely eaten by grasshoppers and covered with black (sugar) ants, feeding on resin extruded from the stem, petioles and leaf bracts. It appears that the high yields were responses to insect damage that induced defense chemicals. Adams et al. (2017b) found that plants grown in a greenhouse from seeds from the high yielding populations (GT, LT) had low to very low yields of HC in lush, protected greenhouse conditions. This seems to confirm the theory of bio-induction of defense chemicals. Additional research is needed to quantify and understand the induction of higher HC yields in *H. annuus*.

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Table 1. Yields of hydrocarbons (HC) *H. annuus*, from natural populations. Coefficient of variation computed as standard deviation / mean.

popn id, sample ids	population sampled	weight 8 lvs	% yield corr'd*	Coef. of variation	Range of yields	yield g/8 lvs
AZ	15021 Camp Verde, AZ	4.48	3.79	33.2%	(1.72-5.56)	0.170
BM	15274 Bozeman, MT	3.28	3.80	25.3	(2.82-5.05)	0.030
BU	15022 Brigham City, UT	5.70	5.90	31.2	(2.90-8.31)	0.336
CM	15287 Clay Co. MN	10.47	2.61	11.2	(1.92-2.95)	0.273
CN	14981 Capulin, NM	3.51	5.29	28.0	(3.16-8.34)	0.186
DK	14940 Dodge City, KS	9.75	3.52	26.2	(2.68,5.49)	0.346
EK	14941 Ellsworth, KS	18.74	5.38	24.0	(3.63,7.35)	1.008
EN	14980 Eagle Nest, NM	4.34	2.62	32.6	(1.17-3.85)	0.114
ENV	15266, Eureka, NV	4.84	3.54	25.3	(2.74-5.56)	0.171
EO	14943 Enid, OK	17.60	4.97	23.9	(3.23,7.55)	0.875
EU	15332 Enterprise, UT bulk,	6.72	5.28	na	na	0.355
FC	14977 Falls Co., Satin, TX	9.49	5.29	14.2	(4.19-6.59)	0.502
FN	15289 Fargo,ND	9.56	4.87	9.3	(4.33-5.77)	0.466
FNV	15265 Fallon, NV	2.17	6.86	28.1	(5.21-11.74)	0.147
GN	15288 Grandin, ND	7.36	3.23	9.1	(2.74-3.71)	0.238
GT1	14952-1 Gruver, TX	16.93	7.26	24.4	(5.01-11.06)	1.231
GT2	14952-2 Gruver, TX	18.03	7.92	19.8	(6.25-10.78)	1.229
GT3	14952-3 Gruver, TX	12.50	8.16	16.4	(7.0-10.51)	1.428
GT4	14952-4 Gruver, TX	14.50	8.60	23.5	(6.52-12.63)	1.020
GU	15262 Glendale, UT	2.95	5.30	44.5	(2.27-10.03)	0.202
HC	14979 Hill Co., TX (Waco area)	5.21	4.92	37.2	(2.61-8.65)	0.263
KU	15270 Kolob Canyon, UT	4.67	3.77	17.0	(2.59-4.53)	0.176
LC	14978 Limestone Co., TX (Waco area)	6.13	3.58	31.3	(2.61-6.25)	0.219
LE	15268, Leeds, UT	6.10	7.01	20.4	(5.7-9.68)	0.428
LT1	14947-1 Lake Tanglewood, TX	16.65	8.47	22.2	(6.73-12.15)	1.410
LT2	14947-1 Lake Tanglewood, TX	13.92	7.31	16.3	(5.63-9.06)	1.018
LU	15023, Logan, UT,	7.57	5.44	25.7	(3.98-8.67)	0.412
MC	14976 McLennan Co., TX, Waco	11.95	6.18	14.4	(4.74-7.76)	0.739
MCU1	15026 Mill Creek, UT 2016	9.54	5.74	26.6	(3.91-8.22)	0.548
MCU2	15273 Mill Creek, UT 2017	6.66	5.25	25.1	(3.43-7.21)	0.350
MK	14939 Meade, KS	10.38	5.64	18.6	(3.91,7.21)	0.585
MT	14982 grown from seed ex Montrose, KS, ex PI 413033	8.44	4.91	8.9	(4.65-5.42)	0.414
MM	15275 MSU, Bozeman, MT	4.47	4.84	29.8	(3.68-5.68)	0.047
NU	15271 Nephi, UT	7.38	4.85	27.8	(1.63-4.03)	0.358
OS	14946 Oslo, TX	6.48	5.95	20.3	(4.19-8.17)	0.406
PI	15024, Preston, ID	4.29	6.30	27.8	(3.91-9.34)	0.270
POI	15025, Pocatello, ID	7.99	5.71	16.0	(4.46-7.55)	0.456
PNV	15267 Pioche, NV	4.58	5.20	33.8	(2.74-8.04)	0.238
PT	14935 Post, TX	8.83	4.36	31.7	(2.33,6.63)	0.385
QN	14936 Quanah, TX	14.41	4.32	23.8	(2.88,5.62)	0.623
RN	15029 Reno, NV	2.87	5.11	30.0	(183-3.82)	0.156
RO	15027 Redmond, OR	5.74	4.06	22.6	(3.37-6.28)	0.233
SC	14953 cult. sunflowers, Oslo, TX	12.41	3.20	13.4	(2.75-3.85)	0.397
SL	14951 San Diego, CA, big leaves	9.04	4.68	21.8	(2.75-6.11)	0.432
SS	14950 San Diego, CA, small lvs.	5.83	4.59	29.2	(2.75-7.51)	0.268
ST	14945 grown from seed, ex Sonora, TX, PI413168	11.47	5.19	32.9	(1.99-7.55)	0.595
TN	15264 Tonopah, NV, 1 plant	7.06	6.39	na	na	0.451
TO	14942 Tulsa, OK	13.64	4.56	23.6	(3.16,6.04)	0.622
WO	14944 Woodward, OK, very large, smooth leaves, cult?	19.64	2.62	15.5	(1.92-3.09)	0.515

*correction factor = soxhlet, 6hr extraction/ hexane 18 hr shaker yield = 2.06

Appendix I Population locations. (see Adams et al. 2017, for populations sampled in 2016).
H. annuus:

GN Common in spots e side of US 89, 1mi. s. of Glendale, flowering, few with seed. Sampled 10 plants for HC. 37° 18' 11" N, 112° 36' 09" W., 5700 ft., 24 Aug 2017, Kane Co., Utah, Coll. *Robert P. Adams 15262*

TN Only one plant found, along highway pavement., US 95, in Tonopah, flowering, 38° 04' 40" N, 117° 15' 04" W., 5830 ft, 28 Aug 2017, Nye Co., Nevada, Coll. *Robert P. Adams 15264*

FNV ~100 plants, along canal ditch, w side of US 95, 8.5 mi s of Fallon, flowering, some seeded, 39° 21' 03" N, 118° 46' 59" W., 3820 ft. Lots of black ants on plants. 28 Aug 2017, Churchill Co., Nevada, Coll. *Robert P. Adams 15265*

ENV ~15 plants, in dry ditch, in pinyon-juniper woodland, N side of US 50, 3.5 mi s of Eureka, flowering, some seeded, 39° 26' 07" N, 115° 55' 22" W., 7040 ft. some black ants on plants. 29 Aug 2017, Eureka Co., Nevada, Coll. *Robert P. Adams 15266*

PNV ~30 plants, in dry ditch, in juniper woodland, W side of US 93, 31.3 mi n of Pioche, flowering, some seeded, 38° 19' 45" N, 114° 36' 38" W., 6130 ft. some black ants on plants, 29 Aug 2017, Lincoln Co., Nevada, Coll. *Robert P. Adams 15267*

LE ~30 plants, in dry ditch and on disturbed Hwy Dept. gravel storage, W side of old US 91, 5 mi S of Leeds, flowering, few seeded, 37° 12' 28" N, 114° 36' 38" W., 3055 ft. some black ants on plants, 9 Sept 2017, Washington Co., Utah, Coll. *Robert P. Adams 15268*

KU ~30 plants, along the pavement in the parking lot of Kolob Canyons National monument, flowering, few seeded, 37° 27' 13" N, 113° 13' 34" W., 5070 ft. , 15 Sept 2017, Washington Co., Utah, Coll. *Robert P. Adams 15270*

NU ~20 plants, flowering, few seeded, along the pavement in the parking lot of Owens-Corning Plant, Nephi, 39° 40' 04" N, 111° 51' 07" W., 5922 ft., 15 Sept 2017, Juab Co., Utah, Coll. *Robert P. Adams 15271*

MCU2 ~20 plants, next to sidewalk, flowering and seedlings, multiple branches. Common along sidewalks, Mill Creek, s side of I80 on 2000 E, east side of 2000E., flowering, few seeded, 40° 42' 56.19" N, 111° 50' 01.98" W., 4539 ft., 15 Sept 2017, Salt Lake Co., Utah, Coll. *Robert P. Adams 15273,* (re-collection, see 2016, Adams 15026, Adams et al. 2017)

BM Gravely roadside, adjacent to wet area with cattail, and Maximilian sunflower, near jct. I90 and frontage RD, E of Bozeman. 0.2-0.5 m tall, BZMT1-5, 45° 40' 30.2" N, 111° 00' 29.4" W., 4810 ft., 15 Sept 2017, Gallatin Co., Montana, Coll Matt Lavin sn, Lab Acc. *Robert P. Adams 15274*

MM Weeds in middle of MSU campus, sporadic with western wheatgrass and quackgrass on dry rocky soil, 0.3-0.5 m tall. MSU1-5, 45° 40' 04.5" N, 111° 03' 01.3" W., 4910 ft., 15 Sept 2017, Gallatin, Co., Montana, Coll Matt Lavin sn, Lab Acc. *Robert P. Adams 15275*

CM West of Felton, MN. Roadside ditch, edge of soybean field. Typical population, approximately 40 plants, 47° 05' 18.2" N, 96° 36' 56.7" W., 895 ft., 23 Aug 2017, Clay Co., Minnesota, Coll. Gerald Seiler, sn, Lab Acc. *Robert P. Adams 15287*

GN East of Grandin, ND, Along edge and in soybean field. Typical population, approximately 50 plants, 47° 12' 15.5" N, 96° 50' 47.11" W., 875 ft., 23 Aug 2017, Cass Co., North Dakota, Coll. Gerald Seiler, sn, Lab Acc. *Robert P. Adams 15288*

FN Typical plants near large drainage ditch, scattered along edge for several hundred feet. Large population that has been there for over 40 years. West side of Fargo, 46° 56' 07.7" N, 96° 51' 34.45" W., 896 ft., 23 Aug 2017, Cass Co., North Dakota, Coll. Gerald Seiler, sn, Lab Acc. *Robert P. Adams 15289*

EU Bulk coll. Enterprise, UT, Washington Co., Utah, along UT hwy 219, in Enterprise, ca. 39°34’24” N, 113°43’59 W, 5330 ft., Coll: *Robert P. Adams 15332*